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**Influence of food on the assimilation of selected metals in tropical bivalves from the
New Caledonia lagoon: qualitative and quantitative aspects**

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Abstract

The present study aimed at examining the influence of food quality and quantity on the assimilation efficiency (AE) of metals in two abundant bivalves in the New Caledonia lagoon, the oyster *Isognomon isognomon* and the clam *Gafrarium tumidum*. Bivalves were exposed via their food to the radiotracers of three metals of concern in New Caledonia (^{54}Mn , ^{57}Co and ^{65}Zn) under different feeding conditions (phytoplankton species, cell density, and cell-associated metal concentration). When bivalves were fed *Heterocapsa triquetra*, *Emiliana huxleyi* and *Isochrysis galbana*, AE of Mn, Co and Zn was strongly influenced by the phytoplankton species and by the metal considered. In contrast, when fed one given phytoplankton species previously exposed to different concentrations of Co, phytoplankton-associated Co load had no influence on the AE and on the retention time of the metal in both bivalves. Metals ingested with *I. galbana* displayed generally the highest AE in both bivalve species, except for Mn in clams for which the highest AE was observed for *H. triquetra*. Influence of food quantity was investigated by exposing bivalves to different cell densities of *I. galbana* (5×10^3 , 10^4 or 5×10^4 cell ml^{-1}). As for food quality, food quantity was found to influence AE of Mn, Co and Zn, the highest AE being observed when bivalves were fed the lowest cell density. Overall, results indicate that the two bivalve species are able to adjust their feeding strategies according to the food conditions prevailing in their environment.

Keywords: Mollusks, *Isognomon isognomon*, *Gafrarium tumidum*, New Caledonia, Radiotracer, Feeding

1. Introduction

Changes in coastal ecosystem functioning due to anthropogenic metal inputs is a worldwide issue of concern especially as metals are not biodegradable and enter biogeochemical cycles (Tessier and Turner, 1995). In the coral reef lagoon of New-Caledonia, metal contamination is a critical problem in relation with its extreme biodiversity (Labrosse et al., 2000). Indeed, the lagoon is subject to an increasing environmental pressure imposed by urban development and intensive mining activities. In addition, the use of hydrometallurgic process employing heated and pressured sulphuric acid (lixiviation) has been recently developed in New Caledonia and is expected to be implemented at industrial scale early 2010 (Goro-Nickel, 2001, 2003). Such a process will provide new potential to exploit laterite soils that display lower nickel (Ni) contents than garnierite ores currently used in pyrometallurgic plants, such as at the Société Le Nickel, and will allow recovering the cobalt (Co) as a by-product (Mihaylov et al., 2000; Dalvi et al., 2004). However, the Ni and Co extraction based on lixiviation is an unselective process that may result in additional discharges of by-product metals such as chromium (Cr), iron (Fe), manganese (Mn) or zinc (Zn) (Goro-Nickel, 2001; Baroudi et al., 2003).

Although long lasting contamination exists in New Caledonia (Laganier, 1991; Ambatsian et al., 1997) with high levels of metals reported in coastal marine sediments (e.g. Fernandez et al., 2006), few data on contamination levels in marine organisms and possible local marine ecosystem impairments are available so far in the open literature

(e.g. Monniot et al., 1994; Dalto et al., 2006; Hédouin et al., 2008a,b; Metian and Warnau, 2008; Chouvelon et al., 2009). Therefore, programmes for monitoring possible impact of the land-based mining activities in the New Caledonia lagoon are needed. Such programmes should largely rely on the use of biomonitor species, as already developed and implemented in temperate areas (e.g. US and EU Mussel Watches; see e.g. Goldberg et al., 1983; Warnau and Acuña, 2007; Thébault et al., 2008). Indeed, the main advantage of the biomonitoring approach compared to direct measurement in water or sediment is to provide a direct and time-integrated assessment of the metal fraction that is actually available to the organisms (bioavailable fraction) (e.g., Phillips, 1991; Coteur et al., 2003; Danis et al., 2004; Metian et al., 2008b).

In this context, both experimental and field studies have recently identified the oyster *Isognomon isognomon* and the clam *Gafrarium tumidum* as promising candidates biomonitoring metal contamination in New Caledonia lagoon waters (Metian et al., 2005; Hédouin et al., 2006, 2007, 2008a; Chouvelon et al., 2009).

It is now well established that food is often a dominant pathway for metal bioaccumulation in marine invertebrates and that food composition and/or quantity can strongly influence metal assimilation efficiency (AE) (e.g., Borchardt, 1983; Riisgard et al., 1987; Wang and Fisher, 1999a). Furthermore, feeding processes such as filtration rate are flexible in marine filter-feeding organisms and may be adapted according to the changes in environmental conditions such as food quantity and/or composition (e.g., Widdows and Donkin, 1992; Navarro and Iglesias, 1993). For example, Cd assimilation in the mussel *Mytilus edulis* is inversely related to food quantity (Borchardt, 1983). In the scallop *Pecten maximus*, food is the main bioaccumulation pathway for Ag (~98%) when

diet is composed of Bacillariophyceae phytoplankton whereas dietary contribution drops below 40% when the scallop is fed Prymnesiophyceae phytoplankton (Metian et al., 2008a). Furthermore, heterorhabdic bivalves (those which gills are composed of 2 different filament types) are also able to select the particles that they are ingesting (Ward et al., 1998), which results in a preferential ingestion of nutritionally-rich particles that may also affect metal influx from food (e.g., Bayne, 1993; Wang and Fisher, 1997).

The objective of this study was thus to investigate the possible influence of food quality (i.e. phytoplankton species) and quantity on the assimilation efficiency of three metals of concern in New Caledonia lagoon waters (Co, Mn and Zn) in the oyster *Isognomon isognomon* and the clam *Gafrarium tumidum*. The variations in the feeding conditions that were considered are: (1) the phytoplankton species used as food, (2) the phytoplankton density and (3) the metal concentration associated with phytoplankton. Radiotracer techniques were used to enhance the detection sensitivity of metals and to allow for measuring metal flux at environmentally realistic contaminant concentrations (Warnau and Bustamante, 2007).

2. Materials and Methods

2.1. Collection and Acclimation

The organisms (n = 100 per species) were collected by SCUBA diving in Maa Bay (oysters *Isognomon isognomon*) or by hand-picking in Dumbea Bay (clams *Gafrarium tumidum*) in October 2003. Both locations are located 15 to 20 km north of

Nouméa City, New Caledonia. Body size is known to affect bioaccumulation of metals in marine organisms (e.g., Boyden, 1974; Warnau et al., 1995); hence, according to previous preliminary studies (Metian, 2003; Hédouin et al., 2006, 2008a), only individuals with a shell longer than 70 mm (*I. isognomon*) or a shell wider than 35 mm (*G. tumidum*) were used in the experiments. After collection, clams and oysters clams were shipped to IAEA-MEL premises in Monaco, where they were acclimated for 2 months to laboratory conditions (open circuit aquarium; water renewal: 30% hr⁻¹; salinity: 36 p.s.u.; temperature T° = 25 ± 0.5°C; pH = 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs) simulating the conditions prevailing in the New Caledonia lagoon. During acclimation, bivalves were fed phytoplankton using the Prymnesiophyceae *Isochrysis galbana* (10⁴ cells ml⁻¹). Recorded mortality was lower than 5% over the acclimation period.

2.2. Radiotracers and Counting

Investigated elements (Co, Mn and Zn) were introduced into the experimental microcosms as radiotracers of high specific activity, purchased from Amersham, UK (⁵⁷Co in 0.1M HCl, T_{1/2} = 271.8 d) and Isotope Product Lab., USA (⁵⁴Mn in 0.1M HCl, T_{1/2} = 312.2 d; ⁶⁵Zn in 0.5M HCl; T_{1/2} = 243.9 d).

Radioactivity was measured using a high-resolution γ-spectrometer system composed of three Germanium -N or P type- detectors (EGNC 33-195-R, Eurysis®) connected to a multi-channel analyzer and a computer equipped with a spectra analysis software (Interwinner® 6). The radioactivity of the samples was determined by comparison with standards of known activities and of appropriate geometry.

Measurements were corrected for counting efficiency, background and physical radioactive decay. Counting times were adapted to obtain counting rates with propagated errors less than 5% (Rodriguez y Baena et al., 2006a).

2.3. Experimental Procedures

2.3.1. Testing the influence of Co concentration in food

Isochrysis galbana cells from an axenic stock culture were resuspended into 4 erlenmeyer flasks (light/dark cycle: 12 hrs/12 hrs at 25°C). Each flask contained 500 ml sterile-filtered seawater enriched with f/2 nutrients without EDTA and Si (Guillard, 1975). Flasks were spiked with 4 increasing Co concentrations (0, 5, 50, 500 ng l⁻¹) and phytoplankton was allowed to grow under these conditions for 6 d. Added Co concentrations were realized using increasing amount of Co(NO₃)₂ (synthesis quality, Merck) and a fixed activity of the corresponding radiotracer ⁵⁷Co (2.5 kBq l⁻¹, corresponding to 0.13 ng Co l⁻¹). The range of concentrations selected covers those encountered in the New Caledonia lagoon waters (Fernandez et al., 2002; Goro-Nickel, 2004). After 6 d of incubation, cell density increased from 10³ to 1.5 × 10⁶ cell ml⁻¹. The cells were gently filtered (1 µm-mesh size, Nuclepore® Polycarbonate filters) and resuspended in clean seawater. The radioactivity of the radiolabelled *I. galbana* in each flask was γ-counted before and after the filtration. The radioactivity of algal cells used in feeding experiments was not significantly different among the different flasks, with an average calculated activity of 0.49 ± 0.14 µBq cell⁻¹.

For each added Co concentration, 4 groups of 9 oysters (shell length from 71 to 94 mm) and 4 groups of 9 clams (shell width from 35 to 40 mm) were placed in 4 aquaria containing 16 l of 0.45- μ m filtered natural seawater (close circuit aquaria constantly aerated; other parameters as previously described). Oysters were acclimated for one week to these conditions and seawater was renewed daily. Bivalves from each aquarium were then allowed to feed for 2 hrs on one out of the 4 batches of previously radiolabelled *Isochrysis galbana* (10^4 cell ml⁻¹) (pulse-chase feeding method; see e.g. Warnau et al., 1996b).

Empty shells were placed as control in each aquarium to check for any direct uptake of radiotracers from seawater due to possible recycling from phytoplankton cells during the 2-hr feeding period (Metian et al., 2007). These control shells were radioanalysed at regular intervals of time.

At the end of the feeding period, all organisms were γ -counted and open circuit conditions were restored (water renewal rate: 30% hr⁻¹; salinity: 36 p.s.u.; T° = 25 \pm 0.5°C; pH = 8.0 \pm 0.1; light/dark cycle: 12 hrs/12 hrs). From that time on, all individuals were γ -counted at different time intervals over a 25-d period in order to determine the whole-body depuration kinetics of the radiotracers ingested with food. Throughout the depuration period, bivalves were fed daily for 1 hr non-radiolabelled phytoplankton (*Isochrysis galbana*, 10^4 cell ml⁻¹).

2.3.2. Testing the influence of phytoplankton species

Two batches of 9 oysters (shell length from 73 to 90 mm) and 2 batches of 9 clams (shell width from 35 to 44 mm) were placed in 2 aquaria containing 16 l of 0.45- μ m filtered natural seawater (close circuit aquaria constantly aerated; other parameters as previously described). Clams and oysters were acclimated to these conditions for 1 week (daily seawater renewal) and then fed either radiolabelled *Emiliania huxleyi* or *Heterocapsa triquetra* (10^4 cell ml⁻¹) for 2 hrs (pulse-chase feeding) in order to assess the possible influence of the phytoplankton species on metal assimilation efficiency and retention capacity in the bivalves. Both phytoplankton species occur naturally in several bays of the New Caledonia lagoon where the clams and oysters are living (Jacquet et al., 2006).

For radiolabelling phytoplankton species, experimental approaches conducted on *I. galbana* were applied to the Prymnesiophyceae *E. huxleyi* and to the Dinophyceae *H. triquetra*. Cells from axenic stock cultures were re-suspended in two different erlenmeyer flasks (10^3 cell ml⁻¹), containing 4.5 l sterile-filtered seawater enriched with f/50 for *E. huxleyi* and enriched with f/2 nutrients without EDTA and Si for *H. triquetra* (Guillard, 1975). The two cultures were spiked with 5 kBq l⁻¹ of ⁵⁴Mn, ⁵⁷Co and ⁶⁵Zn, corresponding to 3.6 ng Mn l⁻¹, 25 ng Co l⁻¹ and 60 ng Zn l⁻¹. The cultures were then incubated for 6 d at 25°C (light/dark cycle: 12 hrs/12 hrs). After incubation, the cell densities were 7×10^5 cell ml⁻¹ for *E. huxleyi* and 1.6×10^5 cell ml⁻¹ for *H. triquetra*. The cells were then gently filtered, resuspended in clean seawater and γ -counted as described above (section 2.3.1.). The radioactivity of algal cells used in the feeding experiments was 0.26 ± 0.18 μ Bq cell⁻¹ for *E. huxleyi* and 0.96 ± 0.11 μ Bq cell⁻¹ for *H. triquetra* for

⁵⁴Mn, 2.1 ± 0.8 and 20.8 ± 12.1 $\mu\text{Bq cell}^{-1}$ for ⁵⁷Co and 3.2 ± 1.3 and 3.3 ± 0.1 $\mu\text{Bq cell}^{-1}$ for ⁶⁵Zn, respectively.

Empty bivalve shells were used as controls for possible metal recycling and whole-body depuration kinetics of radiotracer ingested with the food were determined in both bivalve species as described in section 2.3.1.

2.3.3. Testing the influence of cellular density

Three groups of 9 oysters (shell length from 71 to 92 mm) and 3 groups of 9 clams (shell width from 36 to 45 mm) were placed in 3 aquaria containing 16 l of 0.45- μm filtered natural seawater (close circuit aquaria constantly aerated; other parameters as previously described), and acclimated for one week (daily seawater renewal) during which time their food was prepared.

To do this, cells of *I. galbana* from an axenic stock culture were resuspended in an erlenmeyer flask containing 4.5 l sterile-filtered seawater enriched with f/2 nutrients without EDTA and Si. The culture was then spiked with 5 kBq l⁻¹ of ⁵⁴Mn, ⁵⁷Co and ⁶⁵Zn and incubated for 6 d at 25°C (light/dark cycle: 12 hrs/12 hrs). After incubation, the cell density had increased from 10^3 to 1.4×10^6 cell ml⁻¹. Three sub-samples of 58, 115 and 580 ml of the culture were then gently filtered and resuspended in clean seawater. These 3 batches were prepared to obtain final cell density of 5×10^3 , 10^4 and 5×10^4 cell ml⁻¹ in the 16-l exposure aquaria. The radioactivity of the radiolabelled *I. galbana* was measured before and after the cellular filtration. The radioactivity of algal cells ranged from 1.11 to 1.80 $\mu\text{Bq cell}^{-1}$ for ⁵⁴Mn, 0.83 to 1.37 $\mu\text{Bq cell}^{-1}$ for ⁵⁷Co, 2.69 to 4.38 $\mu\text{Bq cell}^{-1}$ for ⁶⁵Zn.

Each group of clams and oysters was then fed for 2 hrs one of the radiolabelled *I. galbana* batches (5×10^3 , 10^4 or 5×10^4 cell ml^{-1}). Whole-body depuration kinetics of the radiotracers ingested with the food were then followed as described in section 2.3.1 and controls (empty shells) were placed in the aquaria for assessing possible radiotracer recycling.

2.4. Data Analysis

Depuration of the radiotracers was expressed as the percentage of remaining radioactivity (radioactivity at time t divided by initial radioactivity measured in the organisms just after the feeding period $\times 100$) (Warnau et al., 1996b; Rodriguez y Baena et al., 2006b).

Depuration kinetics for all experiments were fitted using kinetic models and statistical methods as described by Warnau et al. (1996a,b) and Lacoue-Labarthe et al. (2008). Depuration kinetics were always best fitted by a double-component exponential equation (decision based on F test and ANOVA tables for two fitted model objects):

$$A_t = A_{0s} e^{-k_{es} t} + A_{0l} e^{-k_{el} t}$$

where k_e is the depuration rate constant (d^{-1}), A_t and A_0 are the remaining activities (%) at time t (d) and 0, respectively, and 's' and 'l' are the subscripts for the short-lived and long-lived components. The short-lived component represents the loss of the radiotracer fraction that remains associated with the faeces and is rapidly eliminated with them, whereas the long-lived component describes the loss of the radiotracer fraction that is actually absorbed by the organism and slowly eliminated (Whicker and Schultz, 1982;

Warnau et al., 1996b). The long-lived component allows assessing the assimilation efficiency (AE) of the radiotracer ingested with food ($AE = A_{0l}$). Also, for each exponential component (s and l), a biological half-life can be calculated ($T_{b/2s}$ and $T_{b/2l}$) from the corresponding depuration rate constant (k_{es} and k_{el}) according to the relation $T_{b/2} = \ln 2 / k_e$.

Constants of the models and their statistics were estimated by iterative adjustments of the model and Hessian matrix computation using the nonlinear curve-fitting routines in the Statistica® 5.2.1 software. Differences among the estimated kinetic parameters for the different feeding conditions were tested using comparison tests of the means and possible trends linking metal concentrations to cell densities were assessed using simple linear regression techniques (Zar, 1996). The level of significance for statistical analyses was always set at $\alpha = 0.05$.

3. Results

Depuration kinetics of the radiotracers were followed in the organisms which ingested enough food to display sufficient radioactivity to be accurately counted. Most oysters met this requirement; however some clams displaying very low activities were discarded. No activity was detected on control shells, indicating that no detectable recycling of phytoplankton-associated tracers occurred in the experimental microcosms.

3.1. Effect of Co Concentration in Phytoplankton

Fitting of the whole-body depuration kinetics of ^{57}Co in oysters fed Co-loaded *I. galbana* by a double-exponential model was quite satisfactory (R^2 : 0.86-0.90) for all the food-associated Co concentrations tested (Table 1, Fig. 1). The major fraction (80-85%) of the total radioactivity in oysters was rapidly lost ($T_{b/2s} < 1\text{d}$) whereas the long-lived component accounted for only 15-20% of the ^{57}Co ingested with food that was eliminated with a biological half life ($T_{b/2l}$) ranging from 13 to 25 d.

Similarly, the fit of the whole-body depuration of ^{57}Co in clams was quite good (R^2 : 0.27-0.64) for all the Co concentrations tested (Table 1, Fig. 1). However, the estimated AE of ^{57}Co ingested with food was much higher than in oysters (i.e., 76-84%) and this fraction was retained with a $T_{b/2}$ ranging from 36 to 39 d.

In both bivalve species, no significant difference (p always > 0.05) was found among the estimated kinetic parameters (A_{os} , k_{es} , A_{ol} , k_{el}) determined for the 4 different food-associated Co concentrations.

3.2. Effect of Phytoplankton Species

In oysters, depuration kinetics of ^{54}Mn , ^{57}Co and ^{65}Zn ingested with *I. galbana* (ISO), *E. huxleyi* (EMI) or *H. triquetra* (HET) (10^4 cell ml^{-1}) were best described by a double-exponential model (R^2 : 0.23- 0.63 for ISO, 0.11-0.83 for EMI and 0.57-0.92 for HET) (Table 2, Fig. 2). No significant difference was found among estimated $T_{b/2}$ for all radiotracers and all phytoplankton species tested. In addition, no significant difference was found between AEs for Co and Mn in oysters fed EMI and HET, and for Zn in oysters fed ISO and EMI. In contrast, significant differences ($p < 0.02$) among AEs were

observed for Co and Mn (ISO > HET = EMI, $p < 0.004$) and for Zn (ISO = EMI > HET, $p < 0.02$).

In clams, fitting of the whole-body depuration of the radiotracers ingested with *I. galbana*, *E. huxleyi* or *H. triquetra* (10^4 cell ml^{-1}) were generally somewhat better than in the oyster (R^2 : 0.47-0.98 for ISO, 0.47-0.93 for EMI and 0.61-0.89 for HET) (Table 2, Fig. 2). $T_{b/2}$ of ^{54}Mn was significantly longer when it was assimilated from HET than from EMI or ISO ($p = 0.03$ and 0.004 , respectively). Significant differences were also observed among AEs calculated for Co, Mn and Zn ingested with the three phytoplankton strains (Mn: ISO < EMI = HET, $p < 0.03$; Co: ISO = EMI > HET, $p < 0.0004$; and Zn: ISO \geq EMI \geq HET, $p = 0.04$).

3.3. Effect of Cellular Density

When oysters were fed 10^4 and 5×10^4 cells ml^{-1} of radiolabelled *I. galbana*, whole-body depuration kinetics of ^{54}Mn , ^{57}Co and ^{65}Zn were fitted with R^2 ranging from 0.23 to 0.63 and 0.38 to 0.60, respectively (Table 2, Fig. 3). No significant difference in $T_{b/2}$ and AE between cell densities was found for Co. In contrast, significant differences in AE were found for Mn and Zn, with higher AE calculated at the low cell density ($p = 0.001$ and 0.0003 , respectively).

For clams, examination depuration kinetics of the radiotracers (R^2 : 0.33-0.65 at 5×10^3 cell ml^{-1} and 0.47-0.98 at 10^4 cell ml^{-1}) indicated that $T_{b/2}$ was not significantly different between the two food densities for all three radiotracers (Table 2, Fig. 3).

However, when fed the low cell density, clams incorporated Co, Mn and Zn with significantly higher AE ($p = 0.003, 0.047$ and 0.0003 , respectively).

4. Discussion

During the last two decades, dietary pathway has been increasingly recognized as a major source of contaminant accumulation in marine invertebrates (e.g. Wang et al., 1996; Reinfelder et al., 1998; Wang and Fisher, 1999b). The assimilation efficiency (AE) and retention time ($T_{b/2}$) are the critical parameters in assessing and modelling the dietary uptake of contaminants and numerous studies have been devoted to assess these parameters in different marine organisms (e.g., Wang et al., 1996; Warnau et al., 1996b, 1999; Pan and Wang, 2008). However, as almost a rule in tropical environments (e.g., Phillips, 1991; Chong and Wang, 2000; Metian et al., 2005), only very few data are yet available regarding AE and $T_{b/2}$ parameters for organisms from New Caledonia (e.g. Hédouin et al., 2006, 2007).

Ideally, the concentrations of metals in the tissues of a biomonitor species should reflect those occurring in the ambient environment. This essential criterion has been previously experimentally assessed for *I. isognomon* and *G. tumidum* for the dissolved pathway (Hédouin et al., 2007, *this issue*). Exposures to a range of dissolved concentrations of As, Cr, Co, Cd, Mn, Ni and Zn indicated that, over a realistic range of concentrations, these elements were generally bioconcentrated in direct proportion to their concentration in seawater (*ibid.*). The results presented here are complementary with

these previous studies as they expand the available knowledge regarding metal accumulation in *I. isognomon* and *G. tumidum* to the dietary pathway.

When ingested with phytoplankton previously exposed to a range of increasing Co concentration (up to 500 ng added Co l⁻¹) Co was shown to be assimilated in the same proportion (AE) and retained with similar relative strength ($T_{b/2}$) whatever the food-associated Co concentration was (see Fig.1 and Table1). The experimental conditions were designed to cover the whole range of Co concentrations that can be encountered in New Caledonia waters, from pristine up to extremely contaminated areas (Fernandez et al., 2002; Goro-Nickel, 2004). Similar trends have been previously reported by Chong and Wang (2000) who observed that concentration of Cd, Cr and Zn in sediment had little effect on the assimilation efficiency of sediment-bound metals in the green mussel *Perna viridis* and in the Manila clam *Ruditapes philippinarum*. However, the response to metal concentration variation in ingested food appears to depend on the element as well as on the species investigated. Indeed, whereas AE of Se in the mussel *Mytilus edulis* was not affected by the Se concentration in the ingested diatoms (*Thalassiosira pseudonana*), AE of Zn and Cd did respectively decrease and increased with increasing contamination of the diatom used as food (Wang and Fisher, 1996). Nevertheless, along with data obtained from exposures to increasing dissolved Co concentrations in the same species (Hédouin et al., *this issue*), the present results on Co AE indicate that in the field Co concentrations in both *I. isognomon* and *G. tumidum* would be reflecting the level of Co in their environment, both in the dissolved and particulate phases.

Whereas food quality and quantity were shown to have limited influence on the retention time of Co, Mn and Zn in clam and oyster tissues, metal AE generally differed

according to the feeding conditions. Metals were generally better assimilated when bivalves were fed *I. galbana* than the two other phytoplankton species (*E. huxleyi* or *H. triquetra*). *I. galbana* cells have comparable cell length (c.l.: 4-6 μm) and cell width (c.w.: 2-4 μm) than *E. huxleyi* cells (c.l.: 3-4 μm ; c.w.: 3-4 μm), but are much smaller than *H. triquetra* cells (c.l.: 20-28 μm ; c.w.: 14-18 μm). Hence, the differences and similarities in AE observed among the feeding conditions indicate that phytoplankton size would not be the major factor driving metal AEs in the two bivalves. Bivalves are able to feed selectively on particles of different size and of different nature (various phytoplankton species as well as inorganic particles) (e.g., Newell et al., 1989) and species-related selectivity and/or dietary preferences could at least partly explain the specific differences observed in AE. Alternatively or complementarily, specific difference in metal speciation such as storage of the metal under bioavailable forms in the cytoplasm of the phytoplankton cells (e.g., Reinfelder and Fisher, 1991; Wang et al., 1996; Metian et al., 2008a) could also explain the AE differences that were observed.

Food availability is another key factor that is well known to influence feeding behaviour of filter-feeding bivalves (e.g. Bayne et al., 1987; Bayne, 1993; Pan and Wang, 2008). Generally, filter-feeders can adjust their filtration rate to ambient phytoplankton density and thereby are able to maintain a stable ingestion rate even at high food concentrations (Jin et al., 1996; Dong et al., 2000; Zhuang and Wang, 2004). Although no conclusion on the influence of this adaptive feeding behaviour could be directly drawn from our results, it is clear that food availability notably influenced the AE of the metals examined in *I. isognomon* and *G. tumidum*.

It is nowadays well documented that the dietary pathway is an important contributor to the global bioaccumulation of metals in marine organisms (e.g. Wang and Fisher, 1997; Metian et al., 2008a). Since the present study has shown that the feeding behaviour of *I. isognomon* and *G. tumidum* is influenced by the feeding conditions (quality and/or quantity of food), it is strongly recommended that future studies take into account these parameters so as to refine the prediction of biodynamic models (e.g., Thomann et al., 1995; Metian et al., 2008a; Pan and Wang, 2008). The consideration of such data is also needed to explain bioaccumulation data obtained in the framework of biomonitoring programmes. For example, Bendell-Young and Arifin (2004) demonstrated the influence of mussel feeding behaviour on their predicted tissue concentrations in Cd, especially under conditions of highly variable quantity and quality of suspended particles.

In conclusion, our experimental results suggest that food quality (phytoplankton composition) and quantity (cell density) may play a significant role in the assimilation of metals ingested with food in *I. isognomon* and *G. tumidum*. Because of the major importance of the dietary contribution to global metal bioaccumulation in marine organisms, it is thus recommended to pay great attention to factors influencing AE. This would help refining both bioaccumulation model predictions and interpretation of data from field surveys and biomonitoring programmes.

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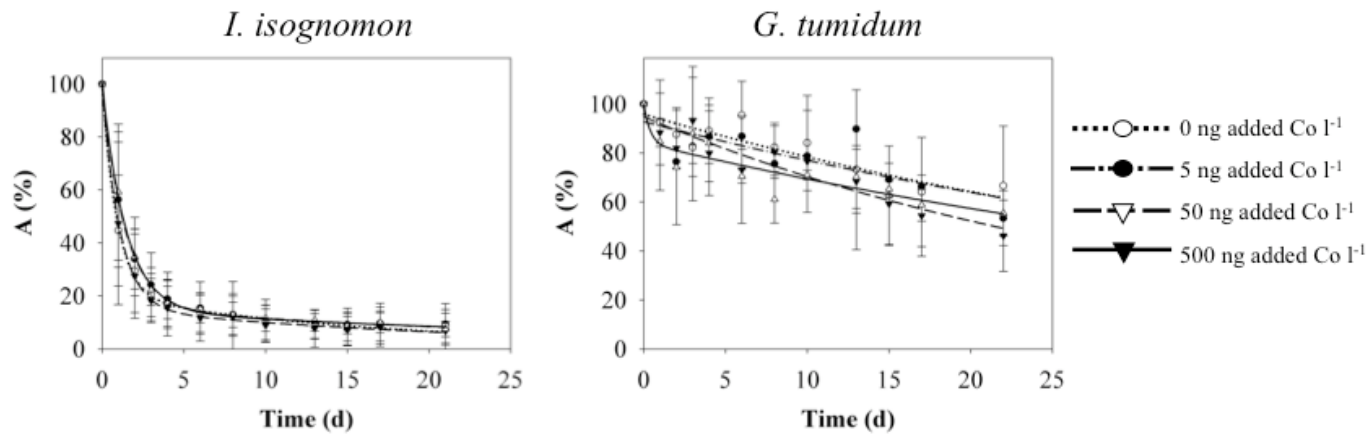
Captions to Figures

Figure 1. Influence of phytoplankton-associated Co concentrations on whole-body depuration kinetics of ^{57}Co in the oyster *Isognomon isognomon* and the clam *Gafrarium tumidum* fed radiolabelled *Isochrysis galbana* (10^4 cells ml^{-1}). A(%): remaining activity (%) \pm SD (n = 9 oysters; n = 6 clams for 0 and 5 ng l^{-1} and n = 8 clams for 50 and 500 ng l^{-1}).

Figure 2. Influence of phytoplankton species (*Isochrysis galbana*, *Emiliana huxleyi* and *Heterocapsa triquetra*; 10^4 cells ml^{-1}) used as food on whole-body depuration kinetics of ^{54}Mn , ^{57}Co and ^{65}Zn in the oyster *Isognomon isognomon* (n = 9 for *I. galbana* and 8 for *E. huxleyi* and *H. triquetra*) and the clam *Gafrarium tumidum* (n = 8 for *I. galbana* and 7 for *E. huxleyi* and *H. triquetra*). A(%): remaining activity (%) \pm SD.

Figure 3. Influence of phytoplankton cell density (5×10^3 , 10^4 or 5×10^4 cells ml^{-1}) on whole-body depuration kinetics of ^{54}Mn , ^{57}Co and ^{65}Zn in the oyster *Isognomon isognomon* (n = 9 for 10^4 cells ml^{-1} and 5×10^4 cells ml^{-1}) and the clam *Gafrarium tumidum* (n = 6 for 5×10^3 cells ml^{-1} and n = 8 for 10^4 cells ml^{-1}) fed radiolabelled *Isochrysis galbana*. A(%): remaining activity (%) \pm SD.

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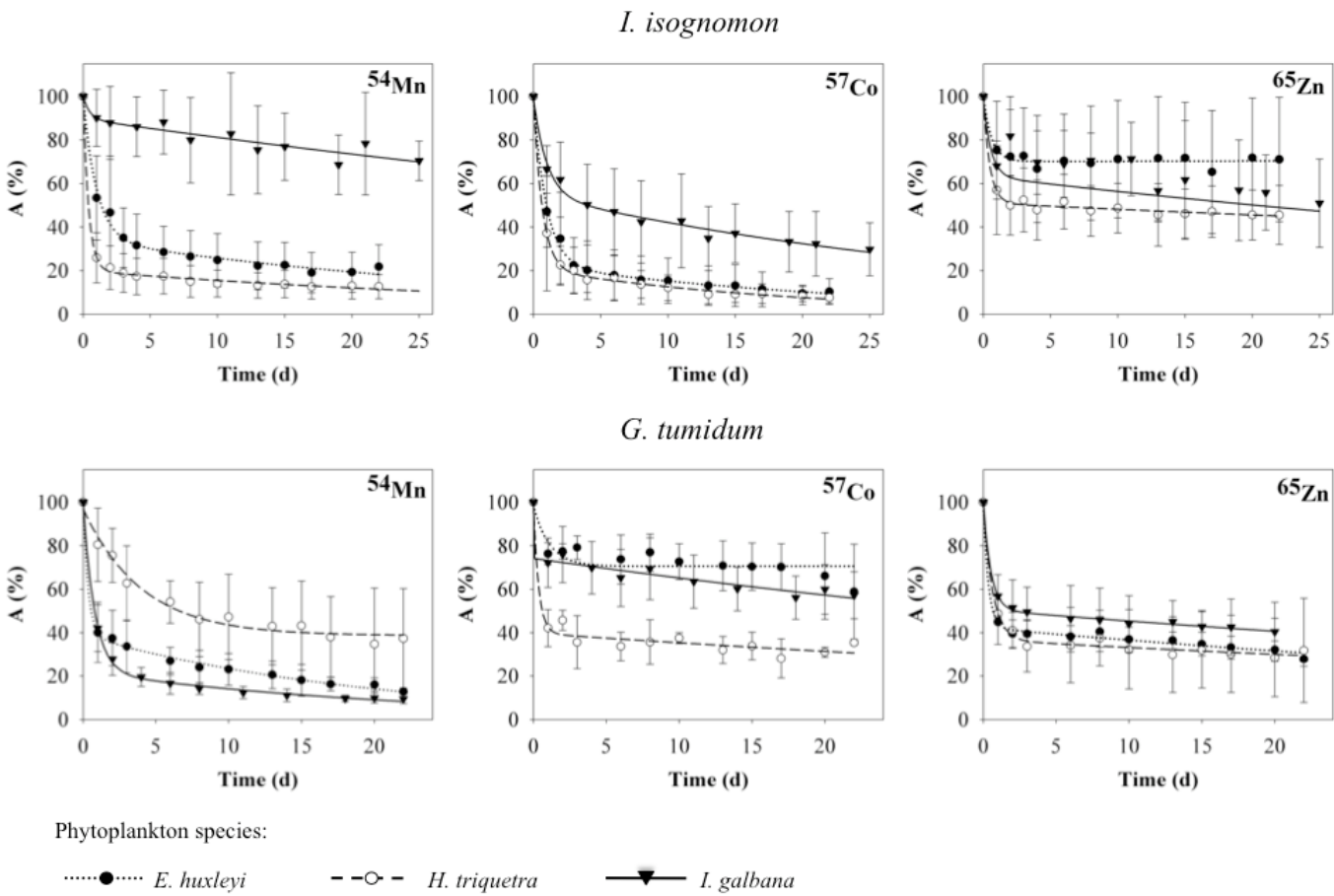
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665 Fig. 1.

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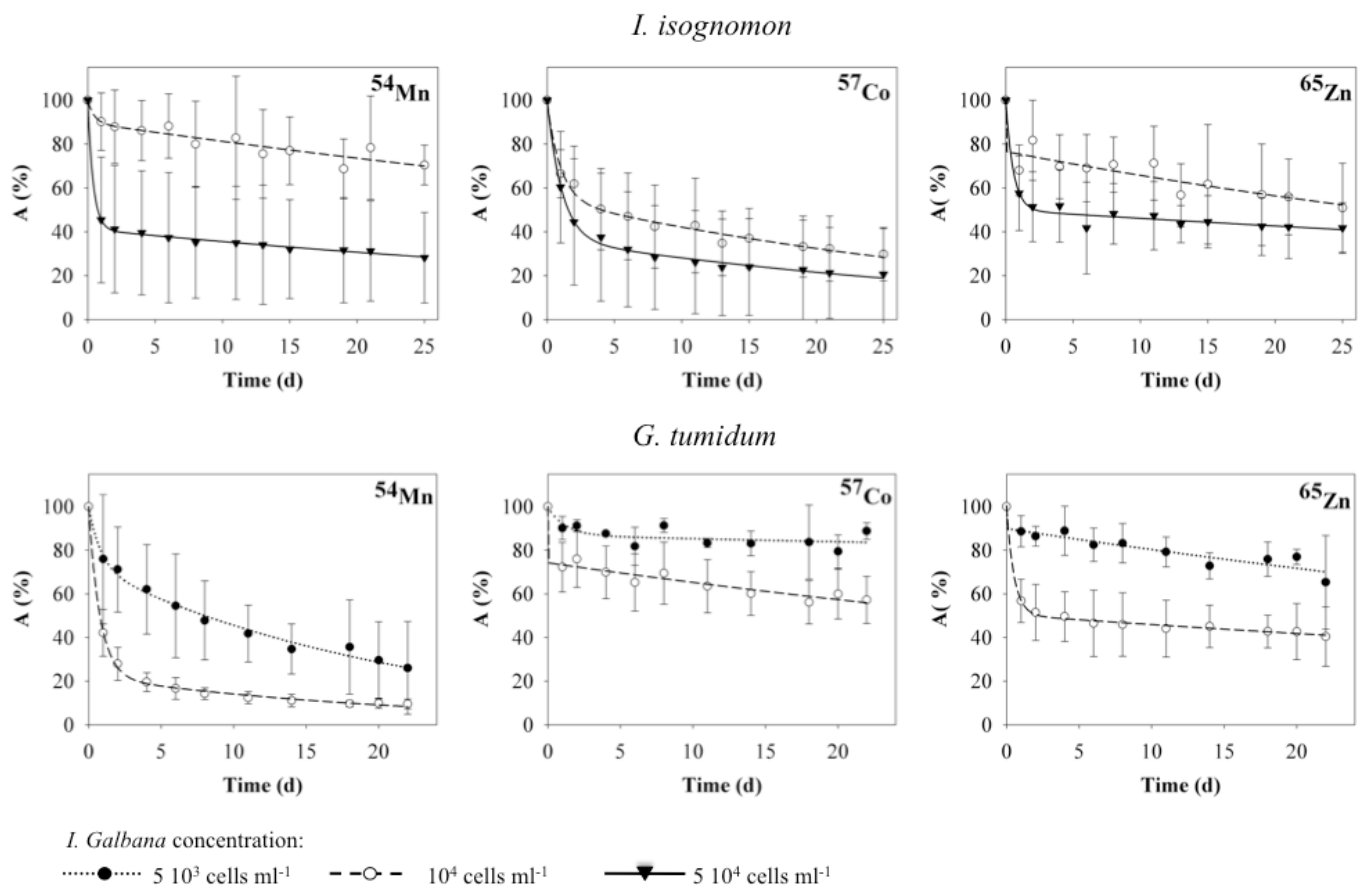


Fig. 3

Table 1. Assimilation efficiency (AE, %), depuration rate constant (k_{el} , d^{-1}) and biological half-life ($T_{b/2}$, d) of ^{57}Co in the oyster *Isognomon isognomon* and the clam *Gafrarium tumidum* fed radiolabelled *Isochrysis galbana* (10^4 cell ml^{-1}) previously exposed to four increasing Co concentrations (n = 9 oysters per concentration tested, n = 6 clams for 0 and 5 $ng\ l^{-1}$ and n = 8 clams for 50 and 500 $ng\ l^{-1}$). ASE: asymptotic standard error; R²: determination coefficient.

Species	Co concentration added ($ng\ l^{-1}$)	AE \pm ASE	$k_{el} \pm$ ASE	$T_{b/2} \pm$ ASE	R ²
<i>I. isognomon</i>	0	15.8 \pm 7.0 ^a	0.032 \pm 0.036*	22 \pm 24*	0.87
	5	19.6 \pm 5.4 ^c	0.054 \pm 0.028*	13 \pm 7*	0.88
	50	16.6 \pm 6.2 ^b	0.050 \pm 0.036*	14 \pm 10*	0.86
	500	14.7 \pm 6.0 ^a	0.027 \pm 0.033*	25 \pm 30*	0.90
<i>G. tumidum</i>	0	77.2 \pm 3.9 ^d	0.018 \pm 0.006 ^b	37. \pm 11 ^b	0.64
	5	77.4 \pm 3.9 ^d	0.019 \pm 0.006 ^c	36 \pm 10 ^c	0.27
	50	75.7 \pm 3.9 ^d	0.018 \pm 0.006 ^b	39 \pm 12 ^b	0.51
	500	84.1 \pm 5.9 ^d	0.019 \pm 0.007 ^a	36 \pm 13 ^a	0.51

Significance of the estimated parameters: ^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$, ^d $p < 0.0001$, * not significant ($p > 0.05$)

Table 2. Assimilation efficiency (AE, %), depuration rate constant (k_{el} , d⁻¹) and biological half-life, ($T_{b/2}$, d) of ⁵⁴Mn, ⁵⁷Co and ⁶⁵Zn in the oyster *Isognomon isognomon* and the clam *Gafrarium tumidum* fed radiolabelled *Emiliania huxleyi* (10⁴ cell ml⁻¹), *Heterocapsa triquetra* (10⁴ cell ml⁻¹) and *Isochrysis galbana* (10⁴ cell ml⁻¹ and 5 10⁴ cell ml⁻¹ for *I. isognomon*; 5 10³ cell ml⁻¹ and 10⁴ cell ml⁻¹ for *G. tumidum*) (n = 8 oysters; n = 7 clams per phytoplankton species tested). ASE: asymptotic standard error; R²: determination coefficient.

Species	Phytoplankton strain	Cell density (cells ml ⁻¹)	Isotope	AE ± ASE	k_{el} ± ASE	$T_{b/2}$ ± ASE	R ²
<i>I. isognomon</i>	<i>E. huxleyi</i>	10 ⁴	⁵⁴ Mn	34 ± 6.5 ^d	0.028 ± 0.015*	24 ± 13*	0.74
		10 ⁴	⁵⁷ Co	22 ± 5.6 ^c	0.039 ± 0.021*	18 ± 10*	0.83
		10 ⁴	⁶⁵ Zn	70 ± 6.5 ^d	0.0002 ± 0.007*	2783*	0.11
	<i>H. triquetra</i>	10 ⁴	⁵⁴ Mn	20 ± 2.6 ^d	0.025 ± 0.012 ^a	28 ± 13 ^a	0.92
		10 ⁴	⁵⁷ Co	21 ± 4.1 ^d	0.050 ± 0.020 ^a	14 ± 6 ^a	0.88
		10 ⁴	⁶⁵ Zn	51 ± 3.2 ^d	0.006 ± 0.005*	123 ± 109*	0.57
	<i>I. galbana</i>	10 ⁴	⁵⁴ Mn	90 ± 5.6 ^d	0.010 ± 0.005 ^a	70 ± 32 ^a	0.23
		10 ⁴	⁵⁷ Co	55 ± 7.1 ^d	0.026 ± 0.010 ^b	26 ± 10 ^b	0.63
		10 ⁴	⁶⁵ Zn	76 ± 4.1 ^d	0.015 ± 0.004 ^b	45 ± 13 ^b	0.36
		5 10 ⁴	⁵⁴ Mn	41 ± 7.8 ^d	0.015 ± 0.014*	47 ± 46*	0.38
		5 10 ⁴	⁵⁷ Co	37 ± 21*	0.027 ± 0.043*	26 ± 42*	0.51
		5 10 ⁴	⁶⁵ Zn	52 ± 3.5 ^d	0.010 ± 0.005 ^a	70 ± 34 ^a	0.60
<i>G. tumidum</i>	<i>E. huxleyi</i>	10 ⁴	⁵⁴ Mn	39 ± 4.3 ^d	0.051 ± 0.011 ^d	14 ± 3 ^d	0.92
		10 ⁴	⁵⁷ Co	80 ± 3.5 ^d	0.010 ± 0.004 ^a	70 ± 26 ^a	0.47
		10 ⁴	⁶⁵ Zn	42 ± 2.3 ^d	0.014 ± 0.004 ^b	48 ± 14 ^b	0.93
	<i>H. triquetra</i>	10 ⁴	⁵⁴ Mn	56 ± 23 ^a	0.021 ± 0.026*	34 ± 10*	0.61
		10 ⁴	⁵⁷ Co	41 ± 3.1 ^d	0.014 ± 0.006 ^a	49 ± 9 ^a	0.89
		10 ⁴	⁶⁵ Zn	33 ± 7.3 ^d	0.005 ± 0.016*	143 ± 474*	0.71
	<i>I. galbana</i>	10 ⁴	⁵⁴ Mn	22 ± 3.7 ^d	0.044 ± 0.015 ^b	16 ± 5 ^b	0.65
		10 ⁴	⁵⁷ Co	73 ± 2.7 ^d	0.010 ± 0.003 ^b	68 ± 21 ^b	0.33
		10 ⁴	⁶⁵ Zn	51 ± 3.8 ^d	0.013 ± 0.006 ^a	55 ± 25 ^a	0.52
		5 10 ³	⁵⁴ Mn	72 ± 17.9 ^c	0.046 ± 0.021 ^a	15 ± 7 ^a	0.98
		5 10 ³	⁵⁷ Co	87 ± 6.1 ^d	0.002 ± 0.005*	416 ± 135*	0.47
		5 10 ³	⁶⁵ Zn	90 ± 3.0 ^d	0.011 ± 0.003 ^c	61 ± 16 ^c	0.68

Significance of the estimated parameters: ^a p < 0.05, ^b p < 0.01, ^c p < 0.001, ^d p < 0.0001, * not significant (p > 0.05)